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Applied nutritional investigation

Ramadan model of intermittent fasting for 28 d had no major effect on body composition, glucose metabolism, or cognitive functions in healthy lean men



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ABSTRACT

Objectives: There has been a parallel increase in the incidence of obesity and diabetes as well as the number of daily meals. However, evidence is lacking regarding the role of intermittent fasting. The aim of this study was to determine the effects of a Ramadan model of intermittent fasting (RIF; 14 h of daytime abstinence from food and drinking) for 28 d on body composition, glucose metabolism, and cognitive function.

Methods: Ten healthy, lean men were included in a nonrandomized, crossover, intervention study. Testing was performed before a control period of 28 d, as well as before and after 28 d of RIF. Whole-body dual-energy x-ray absorptiometry, magnetic resonance imaging of the abdomen, fitness test, oral glucose tolerance test, and cognitive function tests were performed. As secondary outcome, the participants' physical activity and 72-h glycemic responses were monitored 6 d within each of the periods. Dietary intake, appetite, and mood questionnaires also were assessed.

Results: Comparing Δ differences from testing days; body mass index changes from the control period (Δ mean: 0.2 kg/m², 95% confidence interval [CI], −2 to 0.5) and the RIF period (Δ mean: −0.3 kg/m², 95% CI, −0.6 to −0.1) were significantly different ($P < 0.05$). Secondary outcomes within the RIF period showed an increased area under curve (AUC) for hunger accompanied by a reduced AUC for satiety (both, $P < 0.05$), less mean steps per day ($P < 0.05$), and less positive feelings in the afternoon ($P < 0.01$) compared with the control period. No changes were observed in any of the other evaluated parameters.

Conclusions: Free-living participants were able to comply with 14 h of daily daytime abstinence from food and drinking for 28 d with only a minor effect on body mass index and without any effects on body composition, glucose metabolism, and cognitive function.

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experiments. CT and ELM provided essential materials to the study. NHL, AR, and RKM analyzed the data. NHL drafted the manuscript and the figures. NHL, FBB, BKP, and RKM had primary responsibility for final content. All the authors revised the manuscript thoroughly, and all authors read and approved the final version of the manuscript. The authors have no conflicts of interest to declare.

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Introduction

Obesity, a major global health threat, is caused by a chronic imbalance between energy intake and utilization, and is affected by a number of factors [1]. Among these factors, which include reduced physical activity and intake of energy-dense foods, the number of meals per day may play a fundamental role by influencing our sense of satiety and overall caloric intake [2,3].

There is limited scientific research regarding the optimal number of daily meals and the studies that do exist reach opposing conclusions [4–6]. The Danish Health Authority recommends eating three main meals and several small meals per day as part of a healthy lifestyle with the aim of improving control of blood lipids among others [7].

In contrast, an increase in the typical number of daily meals has been paralleled by an increase in the incidence of obesity and diabetes throughout recent decades [8]. One important argument against increased meal frequency is that people tend to increase energy intake per meal, generally in the form of foods with high sugar and fat content [2,9]. In the Nordic countries, for instance, snacking comprises almost 30% of the total daily energy intake [5].

In this context, recent studies suggest that intermittent fasting (IF) has beneficial effects on health [9–11]. In lower eukaryotes, chronic fasting extends longevity partly by reprogramming metabolic and stress resistance pathways [10]. IF in rodents protects against diabetes, cancer, heart disease, and neurodegeneration. There are some discrepancies among studies with regard to the clinical benefits of IF [10,12]. However, in humans it has been reported that IF may reduce hypertension [13,14], asthma [15,16], and rheumatoid arthritis [17], as well as body weight in obese individuals [10,18,19].

Thus, the increasing interest for IF as a dietary strategy represents a potentially attractive alternative to the dogma of high meal frequency as a pathway to health. However, studies that analyzed the health effects of IF on humans in free-living lean participants have identified varied outcomes, potentially due to a wide range of research designs [20,21].

Millions of people undertake 1 mo of IF in observance of the Islamic Ramadan, where fasting takes place during daylight hours from dawn to sunset. In countries near the north and south poles, the fast can last up to 22 h. However, people in these areas are allowed to assimilate the fast period of Mecca [22], where the longest fasting period is ~14 h, depending on the season. Thus, this reoccurring fasting model may constitute an interesting model for studying IF.

Although some studies, including a meta-analysis [23], suggest that IF during Ramadan may lower body weight, fasting blood glucose, and cholesterol levels in men [24,25], other studies have reached directly opposing study results, e.g., the Ramadan fasting is associated with the following:

- Impaired insulin sensitivity and an increase in the adipokine adiponectin [26];
- Improved insulin sensitivity and a decrease in adiponectin [27]; or
- Impaired insulin sensitivity and a decrease in adiponectin [28].

Moreover, a few studies have shown that the Ramadan increased the number of traffic accidents and resulted in a bad mood, slowed reaction time, poor memory, and work performance, as well as increased irritability [29–31].

Although these studies provide valuable data, they have not accounted for confounders like altered sleep–wake cycle, cultural

habits, physical activity, and changes in food intake [32,33]. Therefore, the present study, inspired by the Ramadan IF, is, to our knowledge, the first to assess in both a systematic and controlled setting, the independent effects of 14 h of IF, which we called the Ramadan model of IF (RIF), for 28 d on metabolic parameters and cognitive function in free-living healthy lean men. The objectives of this study were to explore differences between the RIF period and an ad libitum control period on body composition, glucose metabolism, or cognitive functions.

Methods

Participants and the study design

Obtaining consent from study participants

Participants were given both oral and written information about the study before signing a consent form. The study followed the guidelines from the Helsinki Declaration, and was approved by the Danish Ethics Committee of the Capital Region (record number H-3-2011-023), and registered at the Danish Data Protection Agency (record number 2007-58-0015).

Study participants were recruited by means of advertisement in newspapers and via the Internet. Recruitment began in July 2011 and the last follow-up measurements for the 10 participants were completed by June 2012. None of the control or RIF periods was performed during July (summer holiday) or December (Christmas holiday).

Screening

Study participants were their own controls, and were considered eligible if, after 3 d of monitoring (including an assessment of physical activity, heart rate, and diet), they met the following inclusion criteria: ages 18 to 35 y, body mass index (BMI) between 18 and 25 kg/m², a normal medical examination and blood screen, typically consumed three to six meals a day, and were physically active (i.e., taking >8000 steps/d). Exclusion criteria were female sex (to avoid menstrual cycle effects), use of any kind of daily medications, presence of any chronic diseases, alcohol abuse or consumption of more than 14 units of alcohol per week, tobacco smoking (including occasional smoking), and not being of western European descent (to avoid influence on glucose metabolism in a small study group [34]).

Study design

Participants underwent an 8-wk study consisting of two consecutive periods: a 4-wk control period followed by a 4-wk RIF period. In this nonrandomized, crossover, intervention study the participants were used as their own controls. They were not randomized due to biases such as weight loss and altered beneficial eating habits by fasting before the control period. During the control period, each participant maintained his usual eating and exercise habits. During the RIF period, participants abstained from eating and drinking any fluids for 14 h consecutively during daylight hours for 28 d consecutively. Participants were asked to register any break of the fast by date and time. Outside the fasting period, participants were allowed to eat and drink ad libitum (two meals).

Before and after both the control and RIF periods, participants underwent three test modules: before the control period (BCP), after the control period (ACP), and after the RIF period (AIF; Fig. 1) each lasting 2 d with 28 d between. The test modules at time points BCP, ACP, and AIF comprised of weight measurements and blood pressure, 3-h oral glucose tolerance test (OGTT), maximal oxygen intake (VO₂ max) fitness test, cognitive testing, and a whole-body dual-energy x-ray absorptiometry (DXA) scan. Testing was performed after a 12-h overnight fast at the Centre of Inflammation and Metabolism/The Centre for Physical Activity Research Rigshospitalet, Denmark. Participants also underwent magnetic resonance imaging (MRI) scans at time points ACP and AIF at Rigshospitalet, Denmark. Additionally, during 3 d at the beginning (T1 and T3) and 3 d at the end (T2 and T4) of the control and RIF periods, participants were assessed for habitual energy expenditure using the Actiheart accelerometer (Cambridge Neurotechnology Ltd, Cambridge, UK), daily steps using a pedometer (Yamax Digi-walker CW-300 pedometer), 72-h glucose profile using the Continuous Glucose Monitoring System (Medtronic Diabetes Inc, Northridge, CA, USA), dietary intake, appetite, and mood.

Tests from BCP, ACP, and AIF period study days (Δ difference measurements)

Anthropometric assessment

Weight was measured to the nearest 0.1 kg on an electronic scale (Bisco scales, Farum, Denmark), with participants dressed in underwear. Height was measured to the nearest 0.5 cm using a portable stadiometer (HR 001, Tanita Leicester Portable Height Measure, Tokyo, Japan). BMI was calculated as weight (kg)/height (m)². Waist and hip circumferences were measured to the nearest 0.5 cm; waist circumference was defined as the midline between the lowest border of rib cage

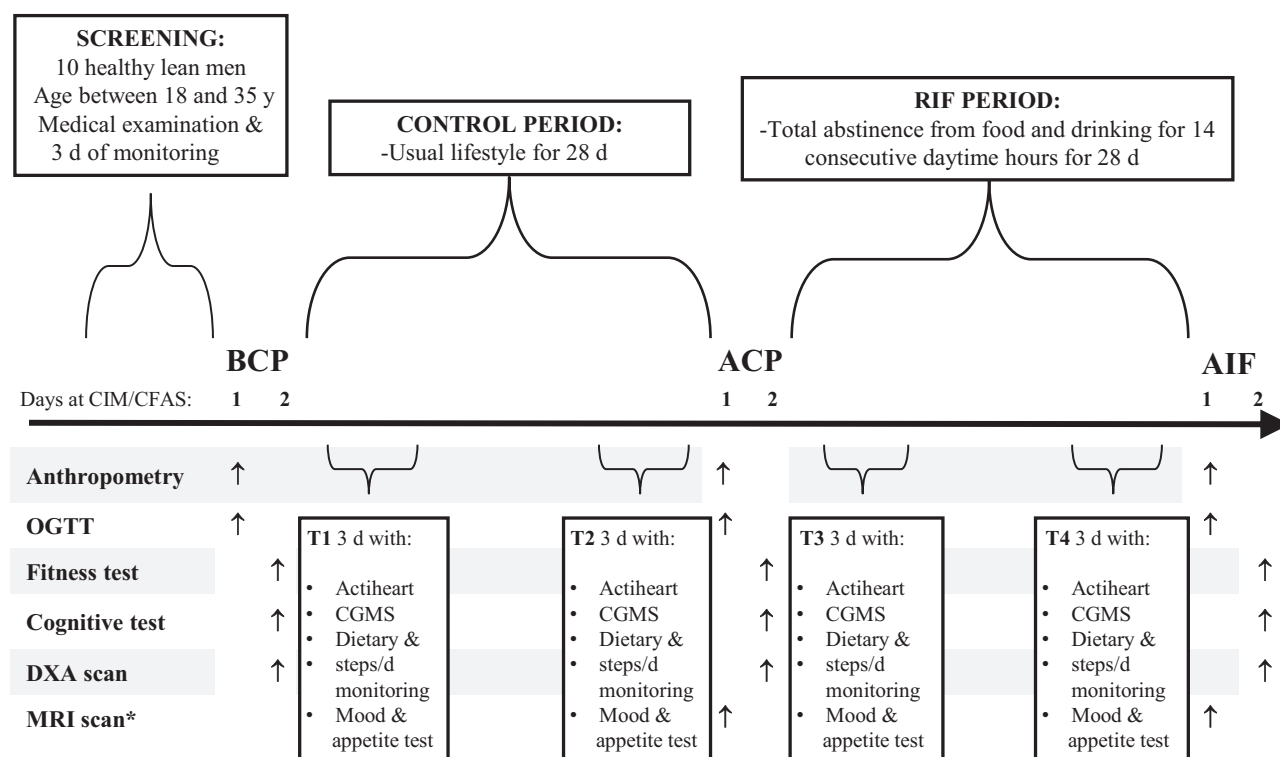


Fig. 1. Overview of the study design. Participants acted as their own control in a nonrandomized, crossover, intervention design consisting of two consecutive periods: A 4-wk control period followed by a 4-wk Ramadan model of intermittent fasting (RIF) period. Arrows indicate day of testing. T1 and T2 are monitoring periods within the control period, and T3 and T4 are monitoring periods within the RIF period. ACP, after the control period; AIF, after the intermittent fasting period; BCP, before the control period; CGMS, continuous glucose monitoring system; CFAS, Centre for Physical Activity Research; CIM, Centre of Inflammation and Metabolism; DXA, dual-energy x-ray absorptiometry; OGTT, oral glucose tolerance test; Actiheart, Actiheart accelerometer monitoring system. *For logistical reasons, magnetic resonance imaging (MRI) scan was only measured before and after the intermittent fasting period.

and the upper border of iliac crest, and hip circumference as the broadest level on hips while the participant was standing, as previously described [35].

Body composition measured by DXA and MRI scans

Total and regional muscle and fat mass of the body was measured using a DXA scan (Lunar Prodigy Advance, GE Medical systems, Milwaukee, WI, USA). For logistical reasons, participants were only scanned twice by 3-Tesla MRI scan (Siemens Magnetom Total imaging matrix magnetic resonance scanner, Erlangen, Germany). The scans were before and after the RIF period from the top of the diaphragm to the pelvic floor. The visceral fat was then double-blinded and manually plotted (on computer using MANGO software version 2.5, University of Texas Health Science Center, San Antonio, TX, USA) on each of the T1-weighted axial images, and volume in mL calculated as the sum of all images.

Standardized OGTT

Participants were asked to register their diet the evenings before the first and second study day of BCP, and subsequently eat exactly the same diet the two evenings before the study days of ACP, and of AIF, respectively. One intravenous catheter was placed in the anterior cubital region after a 12-h overnight fast. After the venous blood baseline samples were drawn, the participant drank a glucose solution consisting of 75 g of glucose (water-free) dissolved in 300 mL of water, and continued to rest for 3-h while 3 mL of blood was drawn every 20 min. Plasma samples were collected in EDTA-containing tubes and immediately centrifuged for 15 min at 3500g, and the plasma was stored at -80°C until further analysis. Serum samples were collected in serum collector tubes and placed upright at room temperature for 20 to 40 min before being centrifuged for 15 min at 3500g and stored at -80°C until further analysis.

Matsuda insulin sensitivity index and homeostasis model assessment-estimated insulin resistance (HOMA-IR) index were calculated as previously described [35].

Cognitive function testing

Participants underwent a battery of neuropsychological tests for memory, executive functions, and attention. A habituation test was performed on the screening day. The testing was performed under optimal conditions (i.e., appropriate lighting, silence, and isolation from unnecessary stimuli). The test battery was comprised of following validated tests: the Trail Making Test A and B [36],

a Danish modified version (only 15 words were read for a total of four times) of the Rey Auditory Verbal Learning Test [37], the Symbol Digit Modalities Test (90-s writing test) [38], and Connors' Continuous Performance Test II (Multi-Health Systems, Toronto, Canada).

Physical capacity assessed by a fitness test

VO₂ max was determined using a standard progressive exercise test on a bicycle ergometer (Monark 839 E, Monark Ltd, Varberg, Sweden). Study participants started with a 5-min warm-up at a workload calculated from body weight and expected VO₂ max, followed by resistance increase in workload every minute until they were unable to maintain a cadence on 60 rpm, VO₂ reached a plateau, or the respiratory exchange ratios were >1.1. VO₂ was continuously measured by indirect calorimetry (Quark b², Cosmed, Rome, Italy). Before study test days, participants performed a VO₂ max fitness test at screening day for habituation of the test.

Biochemical assays

The circulating levels of plasma insulin, plasma glucose, plasma cholesterol, plasma triacylglycerols, plasma alanintransaminase (ALAT), plasma thyroid hormones, and glycosylated hemoglobin (HbA1c) in blood were measured using standard techniques at the Department of Clinical Biochemistry, Rigshospitalet University Hospital, Copenhagen, Denmark. Plasma leptin, plasma adiponectin, plasma interleukin (IL)-6, plasma IL-10, and plasma tumor necrosis factor (TNF)- α levels were measured using Meso Scale Discovery (MSD) electrochemiluminescence (Meso Scale Discovery, Gaithersburg, MD, USA). Serum cortisol was determined by enzyme immunoassay (R&D Systems, Minneapolis, MN, USA). All samples were run in duplicates and the means were calculated.

Tests within the control period (T1 and T2) and within the RIF period (T3 and T4)
(2- point measurements)

Physical activity level by Actiheart and pedometer

Participants' physical activity was assessed by an Actiheart combined heart rate and uniaxial accelerometers monitor that estimates energy expenditure above rest. The Actiheart consists of two sensors linked by a cord and was positioned horizontally on the left chest wall, one sensor at the fourth intercostal room and the other sensor ~ 10 cm away. The two component parts were

secured to the skin via standard electrocardiograph electrode pads (Telectrode, Bio Protech Inc.) and worn both night and day except while bathing. A pedometer was worn attached to the participant's waistband during waking hours (except while bathing), and the pedometer was reset to zero each evening after the daily steps were recorded in a log provided. The mean number of steps registered on the least active single day (minimum) and the mean number of steps registered on the most active single day (maximum) were calculated for each of the participants in the four periods. To adjust for within-week variation in physical activity the total mean steps and Actiheart outcome for each participant were weighted with five of seven and two of seven for weekdays and weekend days, respectively.

Caloric intake

Study participants recorded their dietary intake in detail using a kitchen scale via the Internet for 3 d (2 weekdays and 1 weekend day) five times (in the screening period and at T1, T2, T3, and T4).

Monitoring of appetite and mood

Participants completed 6 questions regarding appetite on a validated appetite visual analog scale (VAS) and 13 questions regarding mood on a 7-point mood scale.

The appetite VAS score consisted of 100-mm lines, and participants were asked to make a vertical mark across the line corresponding to their feelings from 0 (*not at all*) to 100 (*extremely*) for hunger, satiety, thirst, nausea,

prospective food consumption (i.e., amount of food the participant could eat), and fullness. The 7-point mood scale consisted of seven circles. Participants were asked to make a cross in the circle corresponding to their feelings at the time from 1 (*not at all*) to 4 (*some*) to 7 (*a lot*) for nervous, anxious, blue, annoyed, fatigued, restless, cheerful, relaxed, tense, unable to concentrate, carefree, sluggish, and energetic.

Participants completed the questionnaires five times daily for 3 d (2 weekdays and 1 weekend day) during T1, T2, T3, and T4. The five times during each day were just before breakfast, 30 min after breakfast, at 1500 h, just before dinner, and 30 min after dinner.

Continuous Glucose Monitoring System

As previously described [35], the Continuous Glucose Monitoring System (CGMS; Medtronic Diabetes Inc.) consisted of the iPro2 recorder and the Guardian REAL-time CGM, using the same glucose sensor (Enlite Glucose Sensor, Medtronic Diabetes Inc.). The glucose sensor was inserted just under the skin of the lower part of the abdomen in a painless procedure and covered with a supplied transparent dressing (Tegaderm HP, 3 M Health Care, St. Paul, MN, USA). Participants measured a calibrating blood sugar 1 h after insertion, and subsequently four times daily over 5 d (duration of sensor), using a blood sugar apparatus (Contour, Bayer HealthCare, Tarrytown, NY, USA). Data for analysis were matched for each participant in number of hours and of days (including weekdays and weekend

Table 1
Descriptive data and laboratory variables in the 10 study participants

	Baseline	Control Δ	RIF Δ	Δ difference	Δ P value
Descriptive data					
Age, y	25.2 (22.2–28.2)	–	–	–	–
Total body mass, kg	72.7 (67.8–77.6)	0.6 (–0.6 to 1.8)	–1.1 (–2 to –0.2)	1.7 (0–3.3)	0.050
Systolic blood pressure, mm Hg*	125.4 (117.9–132.8)	1.4 (–5 to 7.7)	–1.2 (–5.7 to 3.3)	2.6 (–7.1 to 12.4)	0.55
Diastolic blood pressure, mm Hg*	71.3 (65.9–76.6)	–2.7 (–6.9 to 1.4)	1.8 (8.8)	–4.5 (–14.5 to 5.5)	0.33
VO ₂ max, mL·min ^{–1} ·kg ^{–1} *	49.2 (46.8–51.6)	–0.5 (–2.4 to 1.5)	0.6 (–1.7 to 2.9)	–1 (–4.2 to 2.1)	0.46
DXA scan					
Lean body mass, kg	58.9 (54.4–63.4)	0.4 (–0.5 to 1.2)	–1 (–1.8 to –0.1)	1.3 (–0.3 to 2.9)	0.09
Total fat mass, %	15.2 (12.1–18.3)	0.1 (–0.8 to 1.0)	–0.1 (–0.8 to 0.8)	0.1 (–1.1 to 1.4)	0.82
Gynoid fat mass, %	20.6 (17.1–24.1)	–0.2 (–0.8 to 0.5)	0 (–1 to 1)	–0.1 (–1.4 to 1.2)	0.84
Android fat mass, %	21.3 (17–25.7)	0.0 (–1.8 to 1.8)	–0.4 (–1.7 to 0.9)	0.3 (–1.8 to 2.5)	0.73
Oral glucose tolerance test					
Fasting glucose, mmol/L	4.6 (4.3–4.9)	0.3 (–0.1 to 0.6)	0.3 (0–0.5)	0 (–0.4 to 0.5)	0.92
Fasting insulin, mmol/L	37.8 (24.2–51.4)	–8.1 (–23.3 to 7.2)	2 (–4.5 to 8.6)	–10.1 (–28.5 to 8.3)	0.25
Matsuda Index*	26.9 (19.8–34)	–3.3 (–9.3 to 2.7)	–1 (–8.9 to 7)	–2.3 (–14.8 to 10.2)	0.68
HOMA-IR	1.1 (0.8–1.5)	–0.1 (–0.6 to 0.3)	0.1 (–0.2 to 0.3)	–0.2 (–0.7 to 0.3)	0.35
Biochemistry					
HbA1c, mmol/mol*	32.8 (30.3–35.3)	–1.1 (–2.4 to 0.1)	–0.8 (–1.6 to 0.1)	–0.3 (–1.7 to 1.1)	0.59
Total cholesterol, mmol/L	4.1 (3.5–4.6)	0 (–0.4 to 0.3)	0 (–0.4 to 0.5)	–0.1 (–0.8 to 0.7)	0.83
HDL cholesterol, mmol/L	1.4 (1.2–1.6)	0 (0–0.1)	0 (–0.1 to 0.1)	0.1 (0–0.2)	0.20
LDL cholesterol, mmol/L	2.3 (1.9–2.7)	0 (–0.3 to 0.3)	0 (–0.3 to 0.3)	0 (–0.6 to 0.5)	0.86
Triacylglycerol, mmol/L	0.7 (0.5–0.9)	0.2 (0–0.5)	0 (–0.2 to 0.3)	0.2 (–0.3 to 0.6)	0.40
Alanine transaminase, U/L*	20.9 (15.5–26.3)	4.6 (–1.5 to 10.6)	–4.6 (–13.2 to 4.1)	9.1 (–5.4 to 23.6)	0.19
Thyrotropin, $\times 10$ E–3/L	2 (1.5–2.6)	0.2 (–0.2 to 0.5)	–0.1 (–0.6 to 0.4)	0.3 (–0.4 to 1)	0.37
Triiodothyronine, nmol/L	2 (1.8–2.1)	–0.2 (–0.3 to 0)	–0.1 (–0.2 to 0.1)	–0.1 (–0.3 to 0.2)	0.57
Cortisol, ng/L†	73.7 (52.2–95.3)	25.1 (–0.8 to 51)	–8.4 (–32.6 to 15.7)	33.5 (–8 to 75)	0.10
TNF- α *	4.6 (4.1–5.1)	0.4 (–0.4 to 1.1)	–0.2 (–0.8 to 0.3)	0.6 (–0.7 to 1.9)	0.32
IL-6*	0.3 (0.2–0.4)	0.1 (–0.1 to 0.2)	0 (–0.1 to 0.1)	0 (–0.2 to 0.2)	0.95
IL-10*	2.8 (2.1–3.5)	0.1 (–0.3 to 0.5)	–0.2 (–0.6 to 0.3)	0.3 (–0.4 to 1.0)	0.41
Adiponectin, pg/mL*	14 406 (11 262–17 551)	2761 (91–5431)	–980 (–4324 to 2364)	3741 (–2092 to 9574)	0.18
Leptin, pg/mL*	1434 (499–2369)	997 (–916 to 2910)	604 (–2762 to 3971)	392 (–4694 to 5478)	0.86
Cognitive tests					
Trail Making Test A	22.2 (19.6–24.8)	–2.1 (–4.6 to 0.4)	0.2 (–2.5 to 2.9)	–2.3 (–6.3 to 1.7)	0.22
Trail Making Test B	48.5 (41.4–55.6)	4.6 (–2.9 to 12.1)	–3.5 (–17.2 to 10.2)	8.1 (–9.8 to 26)	0.33
Symbol	60.4 (56.4–64.4)	–1.7 (–7.3 to 3.9)	0.3 (–5.5 to 6.1)	–2.0 (–11 to 7)	0.63
RAVLT sum 1–3	27.7 (25.3–30.1)	2.3 (–2 to 6.6)	1.5 (–1.1 to 4.1)	0.8 (–4.9 to 6.5)	0.76
RAVLT 4, words after 30 min	9.1 (7.2–11)	1 (–0.9 to 2.9)	0.4 (–1.4 to 2.2)	0.6 (–2.5 to 3.7)	0.68
CPTII hit reaction time, T score	39.7 (35.9–43.6)	0.8 (–1.3 to 2.9)	0.3 (–1.9 to 2.5)	0.5 (–2.5 to 3.4)	0.72
CPTII omission, T score	52.3 (29.4–75.2)	–0.6 (–4.6 to 3.4)	–2.1 (–10.2 to 6)	1.5 (–3.4 to 6.4)	0.50
CPTII commission, T score	51.4 (43.1–59.7)	0 (–4.6 to 4.4)	0.5 (–3.2 to 4.2)	–0.5 (–6.8 to 5.7)	0.85

Δ difference, difference between the control Δ and the RIF Δ ; BMI, body mass index; Control Δ , Δ changes (control period effect) calculated by “after control period” minus “before control period” values; CPTII, Conner's Continuous Performance Test II; DXA, dual-energy x-ray absorptiometry; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; IL, interleukin; LDL, low-density lipoprotein; RAVLT, the Rey Auditory Verbal Learning Test; RIF, Ramadan model of intermittent fasting; RIF Δ , Δ changes (intervention effect) calculated by “after RIF” minus “after control period” values; Symbol, symbol digit modalities test; TNF, tumor necrosis factor; VO₂ max, maximal oxygen intake

Presented as mean (95% CI) and P value assessed by paired Student's *t* test

* Only nine participants in this analysis.

† Only seven participants in this analysis.

days) within and between the study periods (giving a total of 72 h for each of the periods T1–T4). Days missing >3 h and with less than two calibrating blood sugars were excluded. Hypo- and hyperglycemic episodes were defined as a blood glucose levels <3.3 mmol/L and >11.1 mmol/L, respectively.

Statistical analysis

All analyses were performed as complete-case analyses. A Δ change for the control period (BCP – ACP) and for the RIF period (AIF – ACP) were calculated. The intervention (RIF period) effect was defined as the difference between the absolute change of the dependent variables between the control period and the RIF period (i.e., the difference between Δ changes between ACP and BCP and between AIF and ACP). These differences were compared with paired Student's *t* tests. For the four time measurements (T1–T4), within-period differences were evaluated with paired Student's *t* tests. Data within each period was pooled as no differences were found, i.e., values from T1 and T2 are pooled as within the control period and T3 and T4 values are pooled as within the RIF period. Paired Student's *t* tests were performed between the control and RIF periods. Outcomes were not corrected for multiple testing as outcomes besides primary (i.e., tests on BCP, ACP, and AIF days) was regarded as corroborating outcome. Data are presented as mean \pm 95% confidence interval (CI) and the significance level was set accepted as $P < 0.05$ (two-tailed). Data analyses were performed using Stata13 (StataCorp, College Station, TX, USA).

Results

Participants

Ten healthy lean men participated in, and completed, this nonrandomized, crossover, intervention study (Table 1). They all had normal medical examinations and normal blood screens during all three test modules (BCP, ACP, and AIF) examining for diabetes as well as thyroid, liver, kidney, respiratory, cardiovascular, and hematologic diseases (data not shown).

Anthropometric assessment and blood pressure measurements

Total body weight (Table 1) as well as BMI (at baseline: 22.5 kg/m², 95% CI, 21.2–23.7) were slightly but significantly different between the control period (control Δ ; 0.2 kg/m²; 95% CI, –0.2 to 0.5) and the RIF period (RIF Δ ; –0.3 kg/m²; 95% CI, –0.6 to –0.1; $P < 0.05$). Waist-to-hip ratio (at baseline: 0.9, 95% CI, 0.8–0.9) did not differ between the control period (control

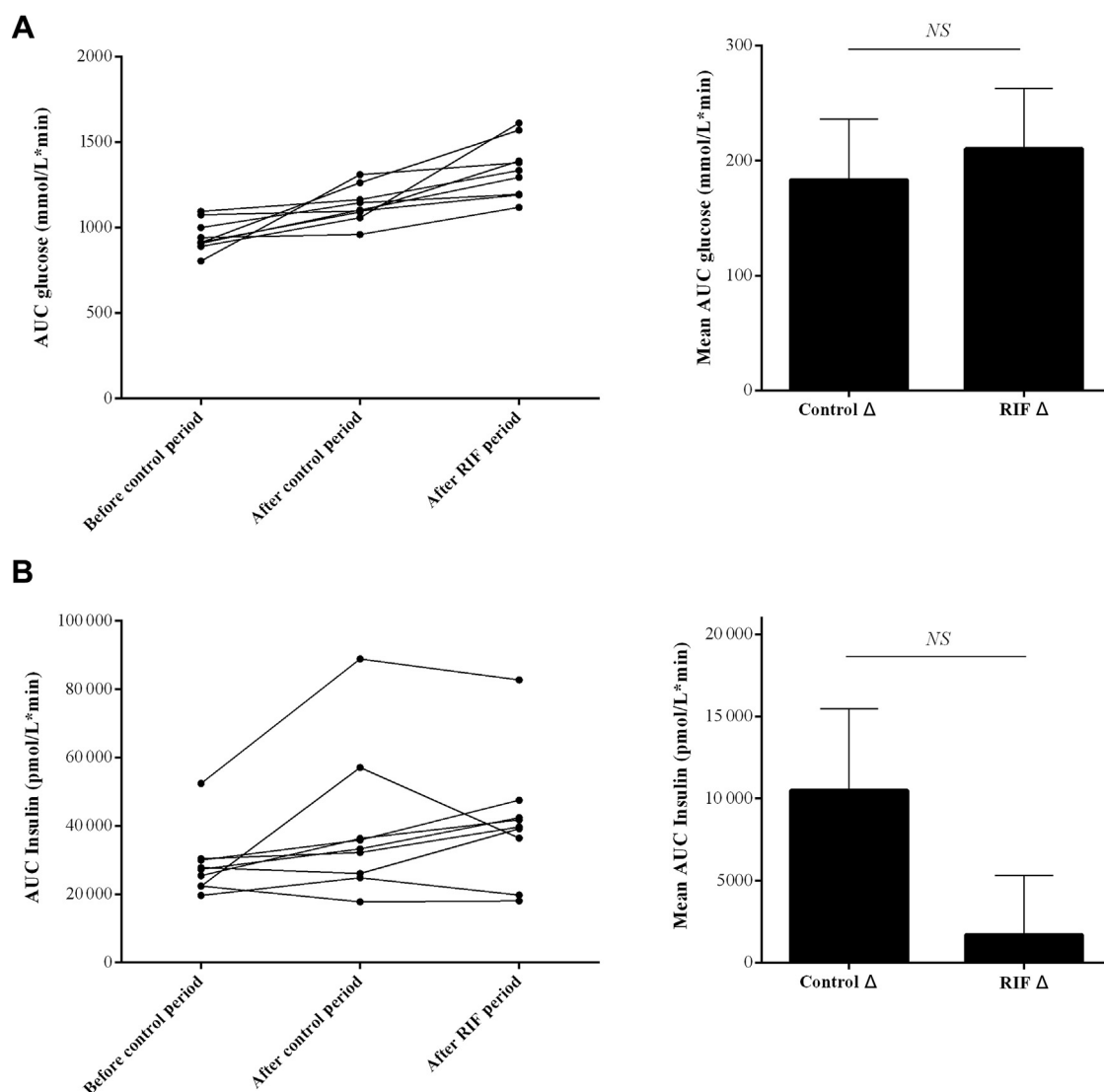


Fig. 2. Glucose metabolism measured by oral glucose tolerance test. The area under curve is shown as individual plots ($n = 9$) at the three time points; before the control period (BCP), after the control period (ACP), and after the RIF period (AIF), whereas Δ changes (i.e., between ACP and BCP and between AIF and ACP) are shown as bar graphs for plasma glucose (A) and plasma insulin (B), respectively. *P* value is assessed by paired Student's *t* test (and SEM as error bars on bar graphs). NS, not significant. AUC, area under the curve; RIF, Ramadan model of intermittent fasting.

Δ ; 0.0, 95% CI, 0.0–0.0) and the RIF period (RIF Δ ; 0.0, 95% CI, 0.0–0.0; $P = 0.42$). Blood pressure changes also did not differ between periods (Table 1).

Body composition measured by DXA and MRI scans

There was no difference between Δ changes with regard to total fat mass, gynoid or android fat mass, or total lean body mass (DXA results; Table 1). Also, MRI scans did not demonstrate a difference for total visceral fat mass determined before (990 mL, 95% CI, 579–1340) and after the RIF period (979 mL; 95% CI, 535–1423; $P = 0.84$).

Standardized OGTT

There was neither a difference between changes in the two periods in fasting plasma glucose and insulin (Table 1) nor in the area under curve (AUC) for glucose and insulin during the OGTT (Fig. 2). The insulin sensitivity determined by Matsuda index and HOMA-IR also showed no differences between changes in the two periods (Table 1).

Cognitive function testing

No differences were observed between changes in performance of memory, executive functions, and attention in the two periods (Table 1).

Biochemical assays and physical capacity assessed by fitness test

There were no significant differences between changes in the two periods with regard to HbA1c in blood, cortisol in serum,

plasma ALAT, plasma thyroid hormones, plasma lipid levels, and in plasma levels of leptin, adiponectin, IL-6 and IL-10, and TNF- α (Table 1). The same was observed for physical capacity (Table 1).

Physical activity by Actiheart and pedometer

Mean number of steps per day were slightly lower in the RIF period than in the control period (Table 2). This finding was not supported by the habitual physical activity monitored by Actiheart, which did not show any differences in any aspects between two periods (Table 2).

Caloric intake and appetite

There were no differences in total caloric or macronutrient intake between the two periods (Table 2).

The total AUC during the monitoring days (T1–T6) presented higher peaks for prandial hunger, thirst, and prospective food consumption scores in the RIF period than in the control period (Fig. 3A–C). In contrast, lower nadirs were observed in the RIF period than in the control period for prandial satiety and (marginally significantly for) fullness scores (AUC fullness for control: 32 398 mm/min, 95% CI, 23 258–41 537 and AUC fullness for RIF: 26 337 mm/min, 95% CI, 19 985–32 688; $P = 0.07$; Fig. 3D, F).

Looking at the mean appetite response in the late afternoon (1500 h), hunger, thirst, and prospective food consumption were higher; satiety and fullness were lower in the RIF fasting than in the control period (Fig. 3). As participants had been fasting for several hours at this time point, this was expected. No differences were found in nausea scores between the two periods (Fig. 3E).

Table 2

Variables from the study days within the control period (T1 and T2) and the 14-h Ramadan model of intermittent fasting period (T3 and T4), $n = 10$

	Control period	RIF period	P value
Energy expenditure			
Pedometer			
Mean weighted steps/d	10 570 (8330–12 811)	9376 (7162–11 590)	0.045
Mean minimum steps/d	5566 (3955–7177)	4685 (3405–5965)	0.23
Mean maximum steps/d	16 039 (12 723–19 355)	15 001 (11 046–18 957)	0.46
Actiheart			
TEE, kJ	12 943 (11 508–14 379)	12 949 (11 499–14 399)	0.98
REE, kJ	7396 (7041–7752)	7391 (7001–7781)	0.85
AEE, kJ	4253 (3163–5342)	4263 (3247–5278)	0.97
Sedentary min, <1 MET	1040 (963–1117)	1027 (940–1114)	0.73
Light min, 1.5–2.9 MET	282.4 (231–334)	299.9 (223–377)	0.60
Moderate min, 3–5.9 MET	94.7 (69–121)	98.6 (74–123)	0.69
Vigorous min, >6 MET	19.3 (11–28)	18 (7–29)	0.72
Energy intake*			
Total energy, kJ	10 912 (9090–12 735)	9965 (7102–12 828)	0.31
Carbohydrate, kJ	3657 (1787–5526)	3536 (1138–5934)	0.80
Fat, kJ	2761 (2158–3364)	2768 (2069–3467)	0.98
Protein, kJ	1704 (1398–2010)	1524 (1181–1867)	0.19
Protein/body weight, g/kg	1.4 (1.2–1.6)	1.2 (0.9–1.5)	0.23
CGMS			
Mean BG, mmol/L	5.1 (4.8–5.3)	5.0 (4.7–5.3)	0.37
Min BG, mmol/L	2.8 (2.5–3.2)	3.0 (2.6–3.4)	0.47
Max BG, mmol/L	8.5 (7.3–9.8)	8.4 (7.4–9.4)	0.70
Coefficient of variation, %	18.3 (13.1–23.5)	16.4 (13.3–19.5)	0.17
Mean BG (mmol/L) 1400 h–1700 h	5.1 (4.8–5.4)	4.9 (4.5–5.2)	0.08
Min BG (mmol/L) 1400 h–1700 h	3.5 (3.1–3.8)	3.5 (3.2–3.8)	0.80
Max BG (mmol/L) 1400 h–1700 h	7.5 (6.5–8.6)	6.7 (5.6–7.9)	0.058

AEE, activity energy expenditure; AUC, area under curve; BG, blood glucose; CGMS, continuous glucose monitoring system; MET, metabolic equivalent; REE, resting energy expenditure; RIF, Ramadan model of intermittent fasting; TEE, total energy expenditure

Presented as mean (95% CI) and P value assessed by paired Student's t test

Control period: pooled data from T1 and T2; RIF period: pooled data from T3 and T4; one MET defined as 3.5 mL O₂/kg body weight per minute; min/max BG is the mean of the lowest/highest blood glucose measured in the time period

* Only nine participants completed the dietary monitoring.

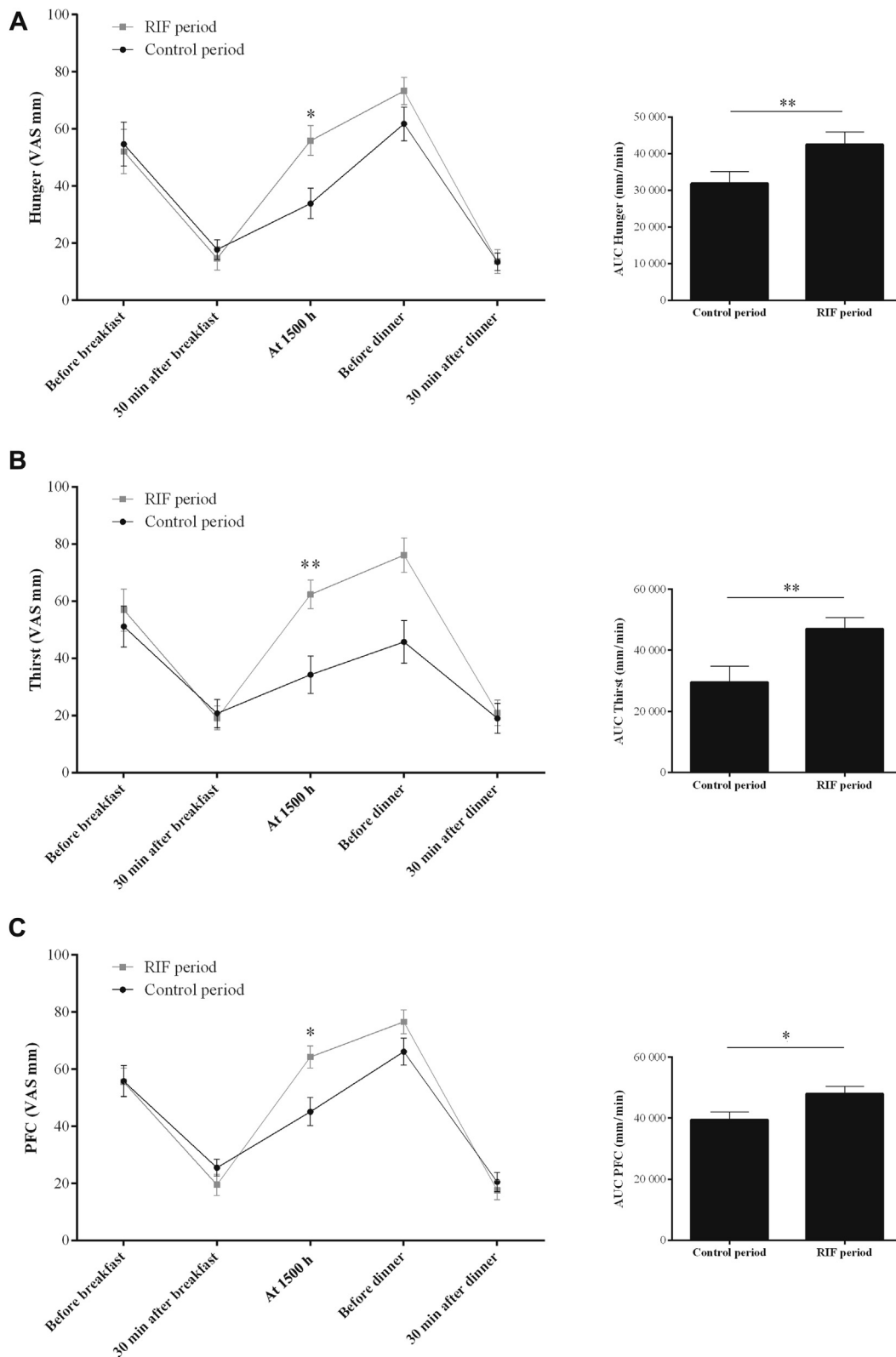


Fig. 3. Appetite score differences assessed by VAS satiety questionnaire. Participants completed VAS questionnaires twice (each time 2 weekdays and 1 weekend day) within the control period and twice within the RIF period ($n = 10$, total of 60 d per period) to determine appetite scores. The figures show the mean VAS response in mm (SEM as error bars) for before breakfast, 30 min after breakfast, at 1500 h, before dinner, and 30 min after dinner with area under curve (AUC) as bar graphs of the six appetite scores: (A) hunger, (B) thirst, (C) prospective food consumption (PFC; i.e., amount of food the participant could eat), (D) satiety, (E) nausea, and (F) fullness. Data are presented as means \pm SE. Paired t tests on the AUC showed the RIF period increased postprandial hunger, thirst, and PFC and decreased postprandial satiety and (marginally significant; $P = 0.07$) fullness. After approximately 6 h fasting (at 1500 h) during the RIF period, participants also showed increased hunger, thirst, and PFC, and decreased satiety and fullness compared with the control period. NS, not significant; RIF, Ramadan model of intermittent fasting; VAS, visual analog scale. * $P < 0.05$. ** $P < 0.01$. No difference where found in nausea scores.

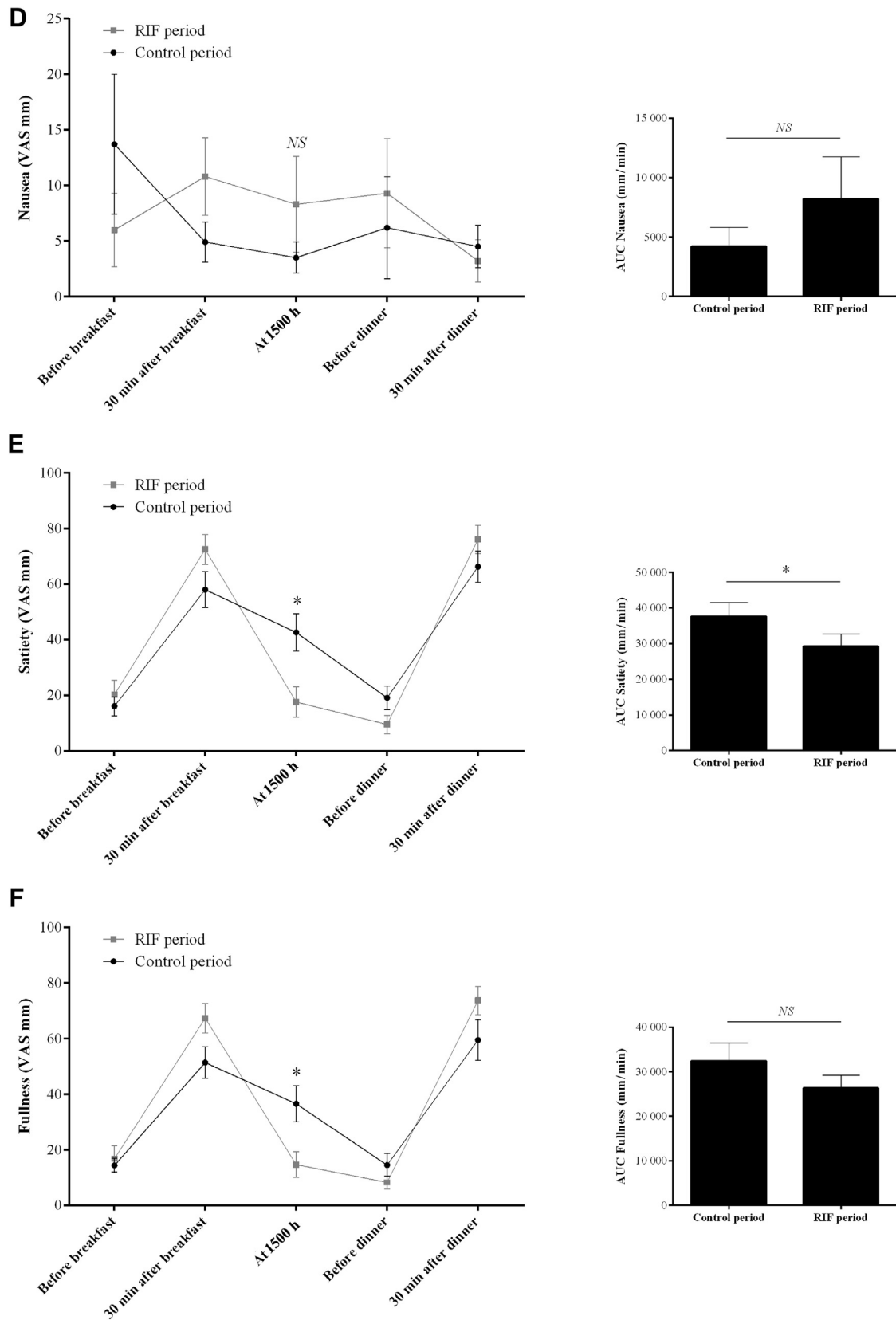
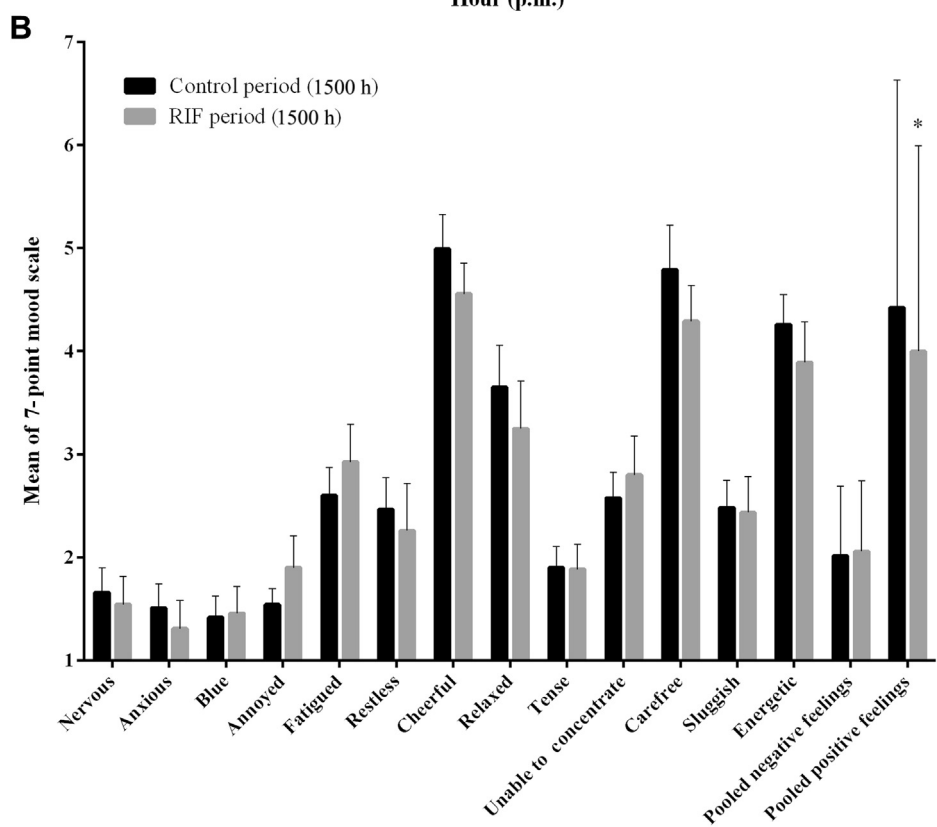
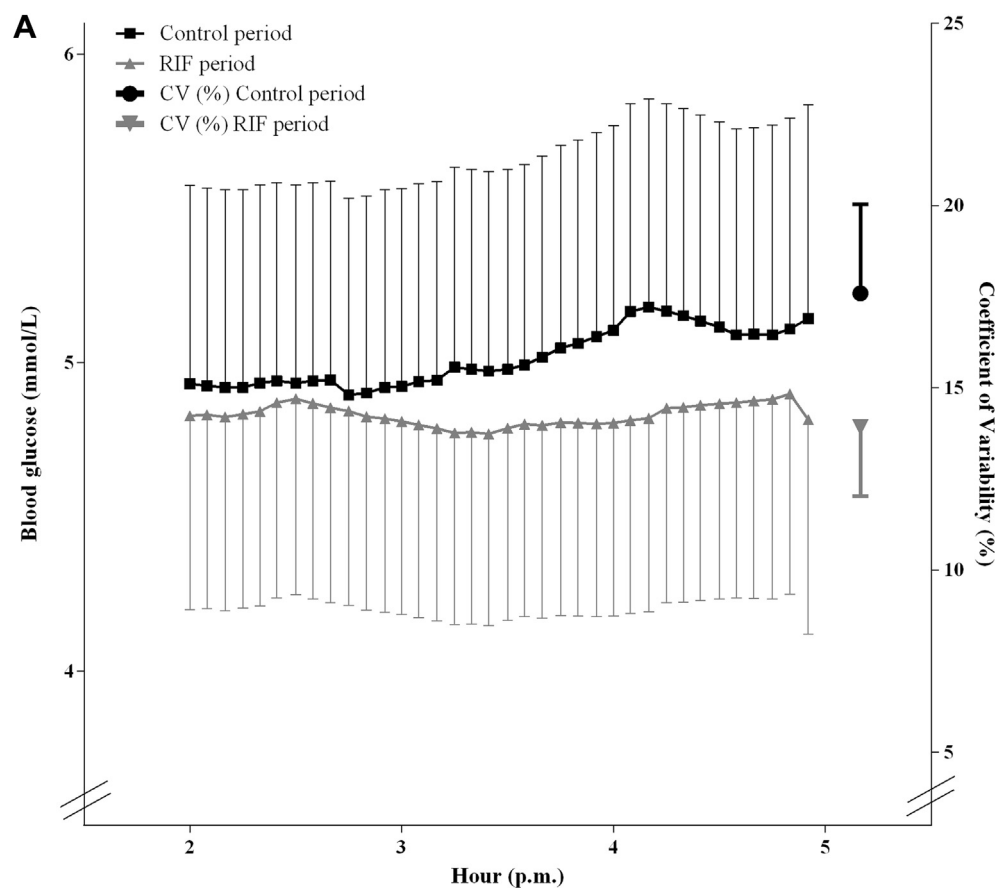


Fig. 3. (continued).



Mood state

The total means of prandial mood response (difference between pre- and 30-min postprandial score for breakfast and dinner) for each feeling was calculated from the 7-point mood scale, to look at possible mood changes if any variation in blood glucose. No differences were found between the control and RIF periods. Looking at the prandial response within the meals in the two periods; pooled pleasant/positive feelings (cheerful, relaxed, carefree, and energetic) were higher postprandially. Hence, in the control period, the pooled pleasant/positive feelings were 4.1% preprandially (95% CI, 3.4–4.7) versus 4.6% postprandially (95% CI, 4–5.2; $P = 0.004$). Within the RIF period the pooled pleasant/positive feelings were 3.8% preprandially (95% CI, 3–4.6) versus 4.3% postprandially (95% CI, 3.5–5.2; $P = 0.007$). At the same time, the unpleasant/negative feelings (nervous, anxious, blue, annoyed, fatigued, restless, tense, unable to concentrate, and sluggish) were lower postprandially. Hence, during the control period, the pooled unpleasant/negative feelings were 2.2% preprandially (95% CI, 1.9–2.6) versus 1.8% postprandially (95% CI, 1.5–2.1; $P = 0.0007$). Within the RIF period the pooled unpleasant/negative feelings were 2.1% preprandially (95% CI, 1.6–2.6) versus 1.7% postprandially (95% CI, 1.2–2.3; $P = 0.0005$), with no observed difference between the two periods (data not shown). At 1500, the above-mentioned pooled pleasant/positive feelings were lower during the RIF period ($P < 0.01$; Fig. 4B).

The CGMS

There were no differences between the two periods with regard to overall mean blood glucose and coefficient of variability (glycemic variability; Table 2). The blood glucose measured with CGMS in the afternoon (between 1400 h and 1700 h) trended toward lower mean blood glucose (5.1 mmol/L in the control period, 95% CI, 4.8–5.4 versus 4.9 mmol/L in the RIF period, 95% CI, 4.5–5.2; $P = 0.08$; Fig. 4A). Furthermore, lower glycemic variability (17.6% in the control period, 95% CI, 12.1–23.1 versus 13.9% in the RIF period, 95% CI, 9.6–18.2; $P = 0.06$; Fig. 4A), and lower maximum blood glucose ($P = 0.06$; Table 2) in the RIF period compared with the control period.

None of the 10 participants experienced hyperglycemic episodes at any time during the control or RIF periods of measurement and there were no differences in the number of hypoglycemic periods between the two periods (data not shown).

Discussion

We presented data from a comprehensive, nonrandomized, crossover, intervention study on the effects of 28 d of 14 h of RIF. In essence, we found very few differences between 28-d periods of RIF and control. Despite a modest decrease in body weight and BMI and a more pronounced hunger feeling accompanied by a reduced satiety sensation, as well as less positive state of mind during the

afternoon during the RIF period compared with the control period, we did not observe any other changes in any of the evaluated parameters. Altogether, the major findings of this study indicate that 28 d of RIF in healthy, lean, fit men do not have an effect on body composition, glucose homeostasis, or cognitive performance.

Our results are partly supported by other studies that found weight maintenance in lean participants after IF [9,39–42]. The conflicting evidence regarding the effects of meal frequency on a number of parameters can be due to several reasons. First, it is essential to remember that the composition of meals (whether carbohydrate-, fat-, or protein-rich) may be of importance [43]. Second, physiological changes that can be measured only after several hours (and days) of fasting can lead to conflicting results [22,29]. Third, the use of different terminology, study designs, and study groups may influence conclusions [5].

The primary findings in the present study may be explained by a steadiness in body composition (i.e., total fat mass, total visceral fat mass, and total lean mass) sustained by a balance in total energy intake (measured by dietary monitoring) and total energy expenditure (measured by Actiheart accelerometer) throughout the study period. Hence, the participants managed to compensate for the lower meal frequency by keeping their overall energy intake in balance. In the abundant different meal frequency studies with diverse health findings in healthy humans, the majority found a significant reduction or increase in total body weight after an IF [11,20]. The independent effect of weight loss due to dietary restriction, exercise, or a combination of the two has been shown in studies to increase positive outcomes in glucose homeostasis, which could also explain the diverse findings in meal frequency studies [27].

Fasting glucose, AUC glucose, and the insulin sensitivity measured by Matsuda index and HOMA-IR from the OGTT had no difference in Δ changes between the two periods. The effect of IF on the glucose metabolism is also disputed, but one recent review supports our findings of no overall effect on glucose homeostasis [20].

Our CGMS data strongly suggest that the nescience in the lay press of one being subjected to a low blood sugar/hypoglycemia leading to mental and physical fatigue in the afternoon (here measured between 1400 h and 1700 h), cannot be accounted for by hypoglycemia in fit lean men, as there were no differences at all in hypoglycemic periods between the two periods. Furthermore, the CGMS data propose (all marginally significantly) lower glucose variability and lower mean, and maximum blood glucose in the afternoon hours during the RIF period. These data also indicate that the participants were truly fasting and further underscores that stable blood glucose can be maintained during fasting in healthy lean men [44].

IF studies in animals have shown improvements in cognitive functions, but human studies are lacking [10]. Although our cognitive tests included several functions (memory, executive functions, and attention), none were influenced by the RIF period. This is in coherence with the rest of our study results, but need to be confirmed by others.

Fig. 4. Afternoon glycemic response and state of mind measured by CGMS between 1400 h and 1700 h and by 7-point mood scale at 1500 h, respectively. Participants ($N = 10$) wore CGMS twice during each period as a control for hyper- and hypoglycemic periods (59 d for the control period and 59 d for the RIF period). (A) The data illustrated are the total mean blood glucose between 1400 h and 1700 h with SEM as error bars. Right side y axis shows coefficient of variability in percent for the time period 1400 h to 1700 h ($P = 0.06$), hence, a trend toward lower glucose variability during the afternoon of the RIF period. The 7-point mood scales were completed five times daily over 3 d during the control (60 d) and RIF periods (60 d). The mood scale consisted of seven circles. Participants were asked to make a cross in the circle corresponding from 1 (not at all) to 4 (some) to 7 (a lot) to 13 diverse feelings they were experiencing at the time. The data showed are (B) total mean scores for each feeling in each period and P value is assessed by paired Student's t test (SEM as error bars on bar graphs). Pleasant/positive feelings (cheerful, relaxed, carefree, and energetic) and unpleasant/negative feelings (nervous, anxious, blue, annoyed, fatigued, restless, tense, unable to concentrate, and sluggish) are pooled in the right end of the bar graph, showing a decrease in the pooled positive feelings at 1500 h during the RIF period compared with the control period. CGMS, continuous glucose monitoring system; CV, coefficient of variability; RIF, Ramadan model of intermittent fasting. * $P < 0.01$.

Participants reported their energy intake and energy expenditure (by Actiheart and pedometer). There was a slight decrease in mean steps per day during both periods, but this decrease was slightly more pronounced during the RIF period. Because the decrease in mean steps was neither supported by Actiheart (or corrected for multiple testing) nor by any change in VO_2 max fitness test, we interpret this (and especially the slightly nonsignificant decrease in energy intake) as a plausible monitoring exhaustion [45], or simply change of physical activity (i.e., biking is not registered with the pedometer). The observed marginal decrease in body weight Δ changes between the control and RIF periods may reflect the observed increase in body weight during the control period, giving a total balanced body weight from start to end of the whole study period. An alternative explanation could be a small dehydration (i.e., the participants abstained from drinking any fluids) supported by a nonsignificant decrease in lean body mass measured by DXA scan. Although the relative weight loss during RIF was not clinically relevant, the possibility exists that RIF over long periods may contribute to maintain a stable low body weight.

A wide range of blood samples (including blood lipids, inflammation, adipokines, and thyroid hormones) supported the primary lack of effect of the RIF period on metabolic and physiologic parameters in the present study. Biochemically, fasting leads to an activation of metabolic mechanisms designed to preserve carbohydrates and increase the dependence on energy produced by the metabolism of fat, which typically occurs after 10 to 12 h of fasting [11]. According to our results, it appears that this shift to ketone bodies during lipid metabolism is safe and does not influence the observed health indicators in lean participants.

The higher fluctuation in appetite sensation (Fig. 3) during the RIF period provides further evidence to the greater peaks and deeper nadirs in appetite sensation, glucose, insulin, and ghrelin found in studies comparing low meal frequency studies to high meal frequency [2,39], although an overall higher AUC hunger and lower AUC satiety sensation were found in the present study in contrast to finding by Munsters and Saris [39]. This could be due to a longer study period and a self-regulation of size, time, and composition of meals in the present study. Our results suggest a lack of habituation to the 28 d of fasting in appetite sensation, which may be necessary to regulate energy intake to maintain weight.

For practical reasons, the mood score test was located at the same time points (prandial and at 1500 h) as the satiety questions. Some studies find an improvement in mood disorders during prolonged (>8 d) fasting with a caloric restriction [46], whereas mood has been found decreased in participants of the Ramadan and women in an IF study [29,47]. In the present study, the pooled positive feelings showed a surprising decrease at 1500 h during the RIF period compared with the control period. Postprandially, during both periods, the participants perceived an increase in pooled positive feelings and a decrease in pooled negative feelings. The presented prandial change in mood is probably experienced as part of the rewarding effects of food intake through the dopaminergic reward system [48], whereas the improvement in mood disorders found during prolonged could be due to the serotonergic system [46]. Thus, the decrease in positive feelings during the afternoon in the RIF period corresponds well with the fact that the participants were eating during the afternoon in the control period and thus had an increase in pleasant/positive feelings postprandially compared with the afternoon during the RIF period, when they were fasting. To our knowledge, few studies have explored the effects on mood (and cognitive functions) during the fluctuations in

hydration of the Ramadan fasting, which also may explain the more negative feelings [33].

Limitations

A randomization of the crossover design would have been preferable with regard to any effect on outcome from different seasons or the likely decrease in monitoring compliance found during the RIF period. However, we considered that weight loss or altered beneficial eating habits introduced by the RIF before the control period would introduce an even more pronounced bias. To prevent seasonal influence the participants were overlapping for the period of 10 mo (i.e., while one participant was in the control period another was in the RIF period). Although several human studies with positive effects from IF are carried out for long-term periods (10–12 mo), some effect from fasting is normally found after 2 to 4 wk [23,27,29]. Obviously, the present data do not exclude the possibility that IF would affect other populations differently (e.g., elderly, women, obese, or individuals who are insulin resistant).

Conclusions

Free-living participants were able to comply with 14 h of daily daylight abstinence from food and drinking for 28 d without any effects on body composition, glucose metabolism, and cognitive function. This suggests that meal frequency may not be as important as overall energy intake with regard to metabolic flexibility in healthy lean males.

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